Scientific Note

Sand fly surveillance and control on Camp Ramadi, Iraq, as part of a leishmaniasis control program

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Vector-borne diseases are a serious health threat to U.S. troops stationed around the world. U.S. forces deployed to Iraq following the 2003 invasion experienced serious risk of infection by several vector-borne pathogens, specifically cutaneous (CL) and visceral leishmaniasis (VL) (Aliaga and Aronson 2007). Camp Ramadi, a U.S. military Forward Operating Base, was established in 2003 at the Al Anbar provincial capital of Ramadi, approximately 110 km west of Baghdad. In Iraq the total number of cases of CL reported per year from 2004 to 2008 was 1,655 and for VL was 1,711 (Alvar et al. 2012). In Al Anbar province in 2008, the estimated incidence of CL and VL was <1 per 10,000 (Alvar et al. 2012). Because of the war, the number of cases of both CL and VL was most likely underreported in Al Anbar and all provinces of Iraq (Alvar et al. 2012).

Because of the risk of vector-borne diseases, each large U.S. military facility, such as Camp Ramadi, had a vector control program operating throughout the year. As part of a base-wide vector control program, unbaited Center for Disease Control and Prevention (CDC) light traps were placed at three locations on Camp Ramadi between April and August, 2009 to gather baseline population estimates of adult sand flies and monitor the success of subsequent control measures. This paper reports sand fly collections over the five-month period, whether they were positive for *Leishmania*, and describes efforts to control sand fly populations using ultra-low volume (ULV) insecticide applications.

Three sites on Camp Ramadi were established for trap placement in areas where U.S. troops worked and lived. Site one was in a grove of palm trees near troop living quarters and the base medical clinic. Site two was in a plot of scrub vegetation near the helicopter landing pad, and site three in tall vegetation near buildings occupied by the base Commander. All three sites had active rodent burrows. Traps were placed 1 m above ground and deployed overnight (from 18:00 until 06:00) approximately every other day. Captured sand flies were taken to the base Preventive Medicine office, frozen, sorted by sex, and counted. Dried specimens were sent to the U.S. Naval Medical Research Unit 3 in Cairo, Egypt, for identification and pathogen testing.

As outlined in the Multinational Force Iraq-I Pest Management Plan, control operations were implemented when the number of sand flies collected in unbaited CDC light traps exceeded a threshold of 15 and when the prevailing wind speed was less than 5 mph as measured by a Kestrel 4000™ (Kestrel Meters, Birmingham, MI) weather meter (Blow et al. 2007). Spray operations were carried out after sunset between 21:00 and 05:00. Two pyrethroid insecticides were used: Scourge® Insecticide (Bayer Environmental Science, Research Triangle Park, NC, U.S.A.) and Anvil® 10+10 ULV (Clarke Mosquito Control Products, Roselle, IL, U.S.A). Scourge® was used from April to June and Anvil® from June to August.

All safely accessible areas of Camp Ramadi had to be sprayed in their entirety as U.S. troops and civilian personnel worked on nearly every part of the base 24 h per day. Therefore, we were not able to establish an untreated area to compare the number of sand flies in sprayed and unsprayed areas. Due to security outside Camp Ramadi, we were unable to access unsprayed areas off the installation.

Trap data were transformed using log (x+1) because there were nights when one or more traps collected no sand flies (Kline et al. 2006). Table 1 provides transformed trap collections before and after ULV applications combined to calculate a mean catch for each night before and after ULV operations. Mean catches, pre-spray and post-spray, and total catches before and after ULV operations were compared with Student's t-test using JMP statistical package (Version 9.02, SAS Inc., Cary, NC).

From April to August, 2009, traps were set 57 times and 6,686 sand flies were collected at the three sites combined, of which 6,366 were identified to genus. For identified sand flies, 1,269 (20%) were from species of *Phlebotomus*; the remainder (5,097, 80%) were *Sergentomyia*. Female *Phlebotomus* spp. composed 10% (n= 636) of the total catch, whereas females of *Sergentomyia* spp. represented 44% (n=2,801) of the total catch. Consistent with seasonal fluctuations of sand fly populations (Coleman et al. 2007), capture rates increased from April to July (April: n=481, May: n=1,109, June: n=1,638, July: n=1,842) and declined in August (n=1,616). Although we did not identify captured sand flies to species level, previous surveys in Iraq indicate three predominant species of *Phlebotomus*: *P. alexandri*, *P. papatasi,* and *P.*

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sergenti (Coleman et al. 2007). All three species are known or suspected vectors of Leishmaniasis in addition to biting pests and should be controlled when necessary (Coleman et al. 2007).

During five months of the sand fly season, 21 spray operations were conducted by Camp Ramadi vector control contract personnel using a Clarke Pro-Mist ULV machine (Clarke Mosquito Control Products, Roselle, IL): April (n=2), May (n=6), June (n=6), July (n=3), and August (n=4). After analyzing the transformed means of trap captures from all three sites combined before and after a ULV application, there was no significant difference between total pre- and post-spray means using Student's t-test (Table 1). Only the pre- and post-spray means on 25 May had a p-value < 0.05.

When we graphed seasonal data as part of a preliminary analysis near the end of the control season, trap collections appeared to be related to something other than our ULV insecticide applications. Based on the observed collection patterns, we suggest that moon phase and illumination influenced trap collections due to the essentially zero cloud cover in Ramadi during the control program. Moon brightness influences trap capture when collecting sand flies with light traps (Santos De-Marco et al. 2002). Figure 1 shows percent moon illumination for Baghdad from March to August, 2009 (www.timeanddate.com; Time and Date AS, Stavanger, Norway) against the total number of sand flies collected each trap night from April to August. During the moon cycles, as percent moon illumination increased, trap catch decreased; trap catch increased with decreasing moon illumination. The amount of time the moon is visible in the night sky, not just brightness, may also influence unbaited light trap catches (Santos De-Marco et al. 2002). Moon illumination and other environmental factors, such as temperature and wind speed, also affect sand fly behavior (Alexander 2000). We did not measure environmental factors during the control season, but such factors cannot be ruled out as possible causes of increased or lowered trap captures (Colacicco-Mayhugh et al. 2011). Because known *Leishmania* reservoirs (jackals, dogs, and rodents) were prevalent on the base, we tested 636 female *Phlebotomus* spp. (grouped into pools) for *Leishmania* DNA using real-time PCR (Villinski et al. 2008). None of the pools were positive for *Leishmania* DNA.

Sand flies in Iraq are most active on warm, clear nights with little wind (Colacicco-Mayhugh et al. 2011), conditions that also are favorable for applying ULV-based adulticides (WHO 2006). Space spraying with ULV is widely used to control sand flies, but few rigorous studies have evaluated its efficacy (WHO 2006). Based on our surveillance program, we do not know whether our ULV missions had any impact on sand fly populations. Coleman et al. (2011) found that various insecticide applications, including ULV, did not have a significant effect against sand flies in southern Iraq.

Table 1. Log (x+1) transformed pre- and post-spray means and standard errors (SEM) of unbaited CDC light trap collections from April to August, 2009 on Camp Ramadi, Iraq. Numbers in bold are significant at p<0.05 using Student's t test.

	Pre-spray		Post-spray		
Date	Mean	SEM	Mean	SEM	p-value
27 April	1.079±	0.247	0.923±	0.283	0.3498
30 April	0.923±	0.283	0.731±	0.409	0.3607
8 May	0.848±	0.087	0.563±	0.282	0.2109
12 May	1.739±	0.095	0.839±	0.422	0.0801
16 May	0.839±	0.422	1.917±	0.196	0.0546
18 May	1.917±	0.196	1.474±	0.197	0.0929
20 May	1.474±	0.197	1.849±	0.269	0.1646
25 May	1.849±	0.269	0.693±	0.158	**0.0152**
9 June	1.219±	0.205	1.616±	0.238	0.1380
12 June	1.616±	0.238	1.419±	0.213	0.2859
14 June	1.419±	0.213	1.788±	0.297	0.1876
19 June	0.987±	0.305	2.002±	0.311	0.9600
21 June	2.002±	0.311	1.380±	0.119	0.0870
26 June	1.684±	0.374	1.310±	0.294	0.2394
6 July	1.445±	0.246	1.055±	0.369	0.2177
26 July	1.934±	0.212	1.505±	0.215	0.1140
28 July	1.505±	0.215	1.423±	0.327	0.4233
6 August	0.890±	0.377	1.066±	0.174	0.3506
18 August	1.751±	0.215	1.697±	0.305	0.4489
21 August	1.697±	0.305	1.509±	0.408	0.3645
26 August	1.848±	0.376	1.834±	0.141	0.4913
Total	1.532±	0.107	1.419±	0.115	0.2387

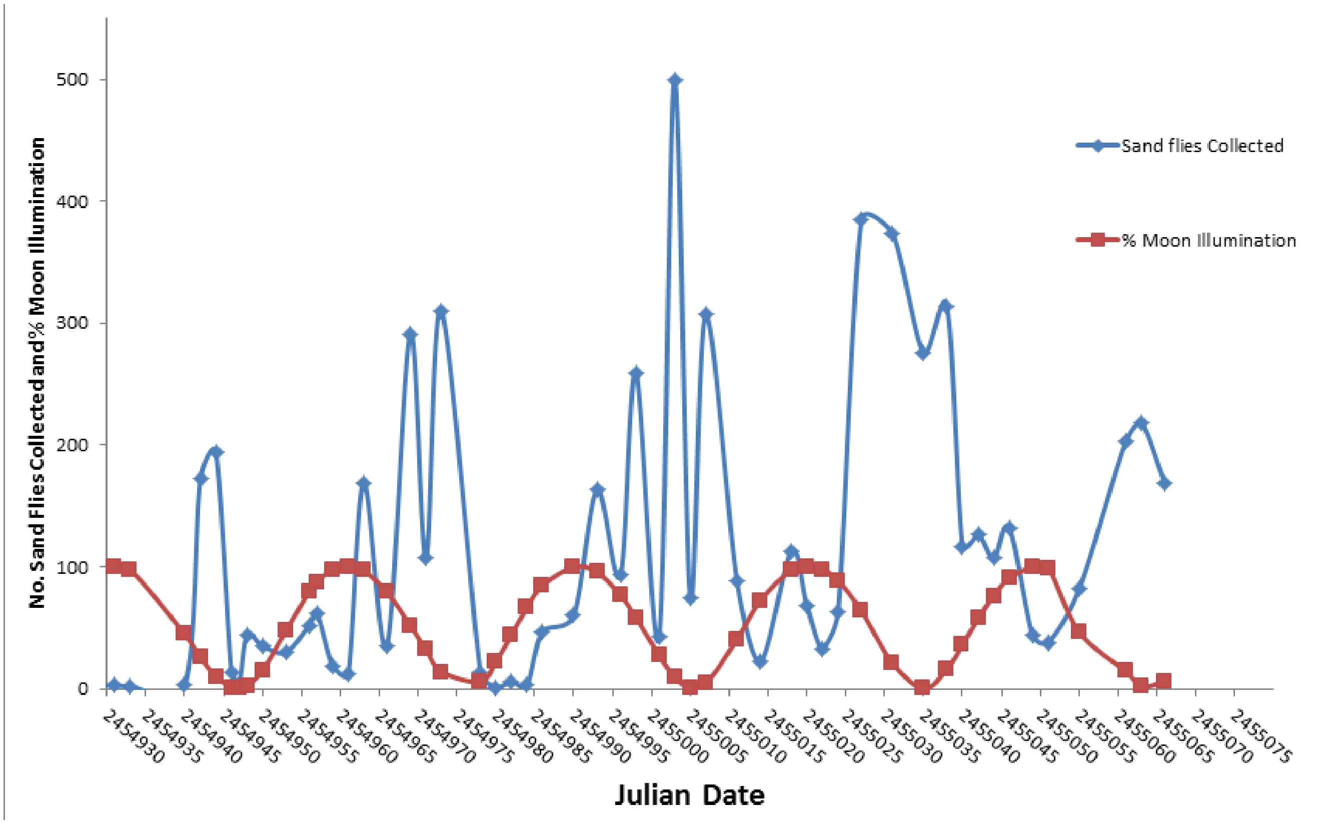

Figure 1. Total number of sand flies collected in unbaited CDC light traps and percent illumination of moonlight from 3 April 2009 to 27 August 2009 on Camp Ramadi, Al Anbar, Iraq.

Based on field trials in Kenya, however, ULV applications of insecticides killed sand flies (Britch et al. 2011). Our insecticide applications were part of an actual vector control program lacking an untreated area to serve as a control, which complicates the interpretation of our results. Because sand flies are biting pests, as well as *Leishmania* vectors, we expected sand fly levels to be near zero following ULV applications. The increasing populations of sand flies, which resulted in peak numbers during July (Figure 1), would have been expected in areas without any control program in place (Coleman et al. 2007). More research is needed to determine the optimal dose and spray parameters for conducting ULV insecticide applications against sand flies. Surveillance programs for monitoring control of sand flies in desert habitats should supplement light trapping with other collection methods (Obenauer et al. 2011).

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